

# On the Y Chromosome Haplotype of the First Farmers in the Historical Territory of Croatia and the Directions of Agricultural Diffusion in Europe

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## SUMMARY

Agricultural production arrived in Croatia's territory from the Middle East at around 6000 BC. The spread of agriculture in Europe, and thus also in Croatia, was spurred by the immigration of populations with the haplotypes Eu4, Eu9, Eu10 and Eu11 (nomenclature according to Semino et al., 2000). The shares (%) of today's inhabitants with these haplotypes are the result of the high number of Neolithic immigrants and the relationship between immigrants and indigenous Palaeolithic inhabitants. The share of "Neolithic haplotypes" in Croatia is lower than in the countries (regions) through which agriculture expanded toward Croatia (Serbia, Kosovo, Albania, Greece, Turkey, Syria and Lebanon). Comparison of the share of "Neolithic haplotypes" in Croatia with the share of such haplotypes in countries north of Croatia (Hungary, Czech Republic, Poland, Ukraine, Belarus and Germany) has shown that the share of Neolithic haplotypes in Croatia does not differ statistically from these countries. This indicates a specific relationship between the indigenous Palaeolithic inhabitants and the Neolithic immigrants in Croatia's historical territory.

## KEY WORDS

first farmers; agricultural diffusion; Y chromosome haplotype

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## INTRODUCTION

Discussion on the links between the origins of agricultural production (Neolithization) in Europe and the genetic structure of Europeans was launched by Ammerman and Cavalli-Sforza (1984) in their book *The Neolithic Transition and the Genetics of Populations in Europe*.

The theories that Ammerman and Cavalli-Sforza put forward spurred a great deal of research and debate (Barbujani et al., 1994; Bentley et al., 2002; Binder, 2000; Chikhi et al., 1998; Harris, 1996; Jurić, 2002; Lillie, 1998; Otte, 1998; Teschler-Nikola et al., 1999). Following our research (Jurić et al., 2001) into agricultural production methods in the oldest agricultural settlements in Slavonia, in the area between Vinkovci and Slavonski Brod, we concluded that land cultivation and animal husbandry technologies that were used in these settlements had emerged and developed in the Middle East and that they were brought to this region of Croatia by immigrants. In that work (sent to print on May 19, 2000), we posited that in the future, when the genetic structure of Croatia's population is determined, analyses would show that the Neolithic immigrants are the ancestors of a large portion of the contemporary population of Croats. The first genetic analyses of samples from Croatia were published only six months after the submission of our work to print (November 10, 2000). In that work (Semino et al., 2000), it was established that haplotypes Eu4, Eu9, Eu10 and Eu11 originate from the Middle East and that they were brought to Europe by immigrants. The authors named these haplotypes "Neolithic markers," while Renfrew (2002) called them "Neolithic haplotypes." These Neolithic immigrants were the first European farmers (Harris, 1996; Gronenborn, 1999; Price, 2000).

Different designations of haplotypes have been used in later research, because the Y chromosome consortium (YCC) established in 2002 proposed a new system of nomenclature and categorisation (Hammer, 2002). The new nomenclature and categorisation encompassed the entire variability of the non-recombinant part of the Y-chromosome by means of a hierarchical classification. The categorisation also includes the divisions in the Eu system according to Semino et al. (2000), which enables a parallel use of both systems. Many authors have adopted this approach, because it is necessary to consider the works published in systems that were used prior to the proposed YCC system if one wants to carry out a research or draw any conclusions. This system set down a method for designation and diversification within the haplotypes of the "Eu" system developed by Semino et al. (2000), and that developed by Underhill et al. (2000 and 2001).

Under the new system, Eu4 is categorized in haplogroup E, Eu9 in haplogroup J, Eu10 in F, and Eu11 in haplogroup G. Subsequently, Semino et al. (2004) conducted additional research into haplogroups E and J, which are more widespread in Europe than the remaining two haplogroups, and published their diversification within haplotypes Eu4 and Eu9.

In the aforementioned work of major importance, Semino et al. (2000) ascertained that the impact of Neolithic immigration on Mediterranean and non-Mediterranean populations was different. This impact was analysed by the course of regression and the extent of correlation between the distance of the analysed populations from Lebanon and Syria (X) and the percentage of "Neolithic markers" in these populations (Y). The analysis is shown in Chart 1.

The analysis by Semino et al. (2000) assumed that there were two directions of the diffusion of agricultural production, and they were named "Mediterranean" and "non-Mediterranean."

However, analyses of the diffusion of agricultural production to the northern shores of the Adriatic Sea indicate that specific relationships existed between immigrant farmers and indigenous inhabitants in this region and that specific pottery cultures emerged (Dimitrijević et al., 1998; Jurić, 2003).

Archaeological research has unambiguously shown that the direction of Neolithization in the territory along the Adriatic coast was different and distinct from the direction that proceeded from Greece toward Pannonia (Benac, 1979). The analyses of the time-line and direction of diffusion of Neolithic pottery cultures confirm the specific aspects of the diffusion of agricultural production along the Adriatic coast.

Study of the origins and diffusion of agriculture and domestication of plants and animals became an unavoidable element of the study of human evolutionary genetics (Jobling et al., 2004), because the shares (%) of haplotypes/haplogroups in various populations of Europeans are to a considerable degree the result of immigration of the first farmers from the Middle East.

This work provides an analysis of the changes in the share (%) of "Neolithic haplotypes"—or the haplogroups according to the YCC—in the three established directions of Neolithization. Testing of the differences in these shares between Croatia and selected countries will contribute to our knowledge on Neolithization in Croatia's territory and also in the territory north of Croatia, i.e. in the area to which agricultural production spread from Croatia's territory.

## MATERIALS AND METHODS

The directions of the diffusion of Neolithization have been determined on the basis of published works on the genetic share of “Neolithic haplotypes” in European and Middle Eastern countries and the appearance and dating of pottery cultures. The share of “Neolithic haplotypes” in these directions has been analysed, and tests have been conducted of the established proportions between Croatia and the following countries or regions: Lebanon, Syria, Turkey, Greece, Albania, Kosovo, Macedonia, Serbia, Hungary, the Czech Republic, Ukraine, Belarus, Poland, Germany, Calabria, Italy (northern), France, Catalonia, the Basque country in France and the Basque country in Spain.

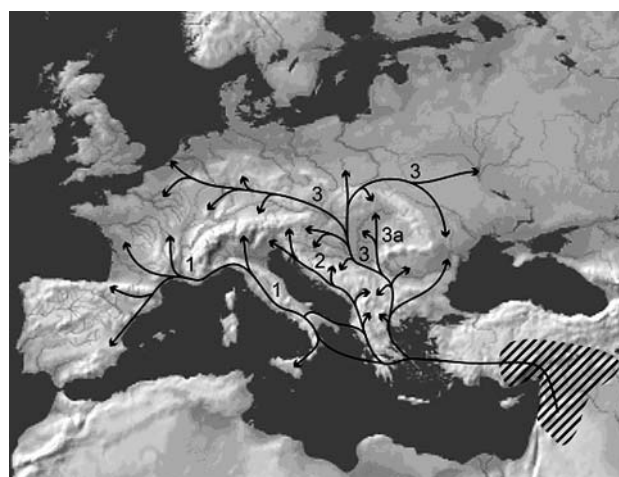
Testing of the differences in proportions has been conducted according to M. F. Triola (2004).

## RESULTS AND DISCUSSION

On the basis of the distribution and changes in pottery cultures, it is possible to ascertain that there were three directions of Neolithization. These directions are designated by numbers 1, 2 and 3 shown on Map 1.

Map 1 shows the three directions of diffusion of agricultural production (Neolithization). Neolithization spread from the Middle East to Greece, and from Greece it expanded to Italy and then to the Atlantic coast on the Iberian Peninsula and in southern France. The second direction of diffusion went along the northern shores of the Ionian and Adriatic Seas to the Alps, while the third went through the Vardar River valley to Central Europe and then to the shores of the Atlantic on the west, and also through western Ukraine toward the Urals.

The divider between the first and second direction of diffusion is the Adriatic Sea, while the divider between the second and third direction is the point of



Map 1. Directions of Neolithization from 8000 to 5000 BC

contact between the Impresso and Starčevo cultures, as established by Benac (1979). The distribution of the Koroš culture is indicated with the direction 3a.

An analysis of the age of finds discovered on the direction of diffusion leading toward the south and north of Croatia is shown in Table 1.

The data in Table 1 make it possible to determine the time of diffusion of agriculture in directions 3 as shown in Map 1.

Table 2 shows the share (%) of the “Neolithic haplotypes,” or the haplogroups based on the YCC system, in the established directions of Neolithization.

The designation accompanying the percentage of “Neolithic haplotypes/haplogroups” pertains to the data sources: 1 – Renfrew, 2002; 2 – Semino et al., 2000; 3 – Peričić et al., 2005; 4 – Kharkov et al., 2005.

Differences from the percentage of haplotypes in Croatia of “Neolithic haplotypes” by directions of agricultural diffusion are shown in Table 3.

Table 1. The results of radiocarbon ( $^{14}\text{C}$ ) dating of some oldest Neolithic sites in Starčevo and Impresso culture (data from Krajcar Bronić et al. 2004)

Sample code	Location	Culture	Conventional $^{14}\text{C}$ age (BP)	Calibrated age (Cal BC) and probabilities
Z-494	Topolčani - Čuka (Macedonia)	Starčevo	7680 $\pm$ 160	6700 – 6370 (61.7%)
Z-495			7010 $\pm$ 190	6060 – 5710 (68.2%)
Z-411	Odmuť (Montenegro)	Neolithic	7440 $\pm$ 150	6440 – 6200 (58.9%)
Z-2924	Zadubravljje – Dužine (Croatia)	Starčevo	7 620 $\pm$ 140	6610 – 6340 (57.2%)
Z-2923			6 995 $\pm$ 115	5930 – 5740 (55.5%)
Z-1967	Vela spila	Impresso	7300 $\pm$ 120	6260 – 6020 (65.0%)
Z-1968	(Korčula Isl., Croatia)		7033 $\pm$ 120	6020 – 5780 (68.2%)
Bln-636	Obre I (Bosnia and Herzegovina)	Impresso	6795 $\pm$ 150	5840 – 5540 (68.2%)
UCLA-1605		Starčevo	6710 $\pm$ 60	5670 – 5550 (60.0%)

Table 2: Neolithic haplotypes according to Semino et al., 2000 and Renfrew, 2002 (Eu4, Eu9, Eu10 and Eu11) or Neolithic haplogroups based on the YCC designations (E, J, F and G) in the directions of agricultural diffusion from the Middle East into Europe

Country (region)	n		%E(Eu4) + J(Eu9) + F(Eu10) + G(Eu11)	Distance from Lebanon and Syria, km
	Total	Neolithic haplotypes		
Lebanon	31	23	74.1 <sup>1</sup>	0
Syria	20	13	65.0 <sup>2</sup>	0
Turkey	30	20	66.5 <sup>2</sup>	650
Greece	76	38	49.9 <sup>2</sup>	900
Albania	51	27	53.1 <sup>2</sup>	1,200
Kosovo	114	73	64.1 <sup>3</sup>	1,200
Macedonia	79	34	43.1 <sup>3</sup>	1,050
Serbia	113	33	29.3 <sup>3</sup>	1,300
Croatia	58	8	13.8 <sup>1</sup>	1,600
Hungary	45	6	13.3 <sup>1</sup>	1,700
Czech Republic	45	7	15.5 <sup>1</sup>	2,000
Ukraine	50	7	14.0 <sup>1</sup>	2,000
Belarus	68	8	11.8 <sup>4</sup>	2,150
Poland	55	2	3.6 <sup>1</sup>	2,100
Germany	16	1	6.2 <sup>1</sup>	2,200
Calabria	37	20	53.9 <sup>1</sup>	1,450
Italy-northern	50	13	26.0 <sup>1</sup>	1,900
France	23	6	26.0 <sup>1</sup>	2,750
Catalonia	24	4	16.7 <sup>1</sup>	2,750
Basque – France	22	1	4.5 <sup>1</sup>	3,050
Basque – Spain	45	2	4.1 <sup>1</sup>	3,400

Table 3. Differences in proportion of “Neolithic haplotypes” between Croatia and other countries (regions) along the three directions of diffusion of agricultural production from the Middle East

Country (region)	“Neolithic haplotypes” Difference from Croatia	“t” size
Lebanon	60.3	+5.6887**
Syria	51.2	+4.4514**
Turkey	52.7	+5.0315**
Greece	36.1	+4.3608**
Albania	39.3	+4.3849**
Kosovo	50.3	+6.2354**
Macedonia	29.3	+3.6751**
Serbia	15.5	+2.2476*
Hungary	-0.5	-0.0734
Czech Republic	1	+0.2426
Ukraine	0.2	+0.0299
Belarus	-2	-0.3361
Poland	-10.2	-1.9082
Germany	-7.6	-0.8234
Calabria	40.1	+4.1803**
Italy-northern	12.2	+1.5974
France	12.2	+1.3094
Catalonia	2.9	+0.3381
Basque – France	-9.3	-1.1755
Basque – Spain	-9.7	-1.6492

\* P < 0.05; \*\* P < 0.01

The results of testing shown in Table 3 lead to the conclusion that the share (%) of “Neolithic haplotypes” in countries (regions) east of Croatia is

significantly higher. It is also higher in southern Italy (Calabria). Along the third direction of diffusion in countries north of Croatia, the share of “Neolithic haplotypes” does not differ statistically from that of Croatia. In northern Italy, France and Catalonia, the decline of “Neolithic haplotypes” compared to the Middle East and Greece and Albania is more gradual, which indicates that the Neolithic immigrants were more numerous or that the original Palaeolithic inhabitants adapted to the new situation more slowly. The differences in the current share of descendents of Neolithic immigrants between the Mediterranean and Central Europe (shown in Chart 1) result from the large decline in “Neolithic haplotypes” in Croatia in comparison to the neighbouring countries east of Croatia. The smaller share of “Neolithic haplotypes” in Croatia’s territory probably influenced the decline of these haplotypes in the territory north of Croatia as well. The difference between Croatia on one hand and Serbia, Kosovo, Albania, Macedonia and Greece on the other, with reference to “Neolithic haplotypes” percentages is statistically significant (Table 3). In this study, P values were not corrected for multiple tests made, for example by Bonferroni correction for experiment - wise  $\alpha$ . Thus, significance obtained should be taken with caution as it was not conservative.

The results obtained in this research allow us to formulate a hypothesis on the specific relationships between Neolithic immigrants, as the first food producers in Croatia’s territory, and the indigenous



Palaeolithic inhabitants. The genetic origin of Croatia's population indicates that the indigenous Palaeolithic inhabitants accepted food production technology rapidly enough to secure their numerical growth, since until the industrial revolution the size of population depended exclusively on available quantities of food.

Research and knowledge on Neolithization in Croatia's historical territory contributes to the understanding of Neolithization in the region north of Croatia, in a broad swath of territory between the Atlantic and Ukraine, that is, in the regions that were home to Linear Pottery cultures.

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